Assessing the adaptation of swine to fifty-seven hours of feed deprivation in terms of behavioral and physiological responses[1]

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ABSTRACT: Dramatic changes in the livestock industry have brought about widespread concern regarding the welfare of animals in terms of the hunger that they may experience. Despite this concern, animal science has not been able to provide a methodology that can objectively determine whether the welfare of animals is compromised by hunger. The current work sought to provide data that characterized the physiological and behavioral responses of animals experiencing 57 h of food deprivation. For this purpose, 2 separate experiments were conducted, in which physiological or behavioral measures were collected from swine deprived of feed for 21 to 57 h or fed normally (physiology, n = 20/treatment; behavior, n = 8/treatment). Treatment × time interactions were found ($P < 0.02$) for insulin, β-hydroxybutyrate, NEFA, drinking, standing, inactivity, lying sternal, lying lateral, and total lying. Animals appeared to adjust appropriately to the metabolic challenge imposed, as suggested by increases in alternative energy substrates (NEFA, β-hydroxybutyrate). Additionally, feed-deprived animals appeared less active than control animals until after 45 h of feed deprivation, when the former appeared to be more active. Our results suggest that feed deprivation of finishing pigs for durations greater than 45 h produced behavioral changes that may be related to increased sensations of hunger and which possibly are dependent on the use of alternative energetic substrates. Our data link behavioral and physiological changes after the imposition of feed deprivation.

Key words: behavior, hunger, physiology, swine, welfare

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INTRODUCTION

Hunger has been identified as a potential welfare issue in a variety of production situations, including restricted feeding in gestating sows (Appleby and Lawrence, 1987; Lawrence et al., 1993) and forced molting in poultry (Cunningham and Mauldin, 1996). Despite efforts to develop objective criteria that determine an animal's welfare in these situations, the subjective nature of stress and problems in quantifying the subjective experience (i.e., hunger) have made identifying animals with compromised welfare a difficult goal to achieve (Hughes and Duncan, 1988; Dawkins, 1990).

A central problem in the effort to identify hunger is the multiple factors that are involved in regulating and resulting from sensations of hunger. For instance, glucocorticoids, commonly released in times of psychological stress, can serve to decrease appetite while enhancing glycogen conversion to glucose (Genuth, 1998). Our lab has been involved in an effort to develop a broad understanding of how various systems that regulate hunger in an individual are altered during periods of increasing feed deprivation and the associated changes in hunger.

The objective of the current project was to develop a profile of physiological and behavioral responses to increasing sensations of hunger imposed by 21 to 57 h of feed deprivation. Given the assumption that animals experiencing longer durations of feed deprivation are hungrier than animals experiencing relatively shorter durations of deprivation, responses from the current study provide a more thorough understanding of the changes induced by feed deprivation and the associated hunger.

MATERIALS AND METHODS

Exp. 1: Physiological Profile

Animals. All procedures involving animals were approved by the Purdue Animal Care and Use Committee.

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Forty group-housed barrows from Purdue University's swine farm were used over 2 replicates (n = 40 barrows; 20 barrows/replicate; 90.0 ± 0.71 kg of BW; Hampshire-Duroc × Yorkshire-Landrace). Barrows were used to eliminate the influence of estrus on feed intake and hunger. The barrows were brought, on d 0, to the USDA-ARS Livestock Behavior Research Unit Farm Animal Behavior Laboratory South facility on the Purdue University campus and randomly assigned to the feed-deprived (n = 10·treatment^{-1}·replicate^{-1}) or control (n = 10·treatment^{-1}·replicate^{-1}) treatments that would be applied on d 8 of the study.

All barrows immediately began a feed regimen in which a standard grower dry diet was accessible from a feed bucket on an ad libitum basis for a 3-h period between 0800 and 1200 h. Feed was provided in this time-limited manner to standardize the hunger experienced across barrows (i.e., after adaptation to the feeding schedule, barrows should have had similar levels of hunger at all times until the treatments were applied on d 8). Barrows had previously been provided free access to the same diet after d 0; thus, a period of adaptation was seen as essential. When the feed bucket was removed each day at the end of the feeding period, all pens were washed, and any excess feed that had fallen from the feed bucket was removed.

On d 9, barrows in the control treatment continued the 3-h, ad libitum feeding routine already described, whereas deprived barrows were not provided feed on d 8 or 9, resulting in a 57-h fast from the time the barrows last had access to feed on d 7 at 1100 h. Fifty-seven hours of feed deprivation was chosen to allow comparison to a wide range of dietary manipulations (i.e., restricted and interval feeding in sows). Barrows were housed individually in pens measuring 1.52 × 1.82 × 0.96 m (width × length × height), with the treatments separated to eliminate visual and tactile contact between barrows of the same treatment. Within treatment, barrows were housed beside each other and thus could see and touch pigs of the same treatment in neighboring pens. Water was accessible at all times. Environmental temperatures were maintained between 18 and 26°C by 2 exhaust fans regulated by a thermostat positioned 2 m from the floor. Lighting was provided between 0700 and 2100 h. Two barrows of the control treatment were excluded from all blood collections due to signs of illness exhibited during the 8-d period of adaptation, including lethargy and lack of eating.

Blood Collection and Catheterization. On either d 4 or 5, jugular catheters were established in each animal with a nonsurgical procedure. Briefly, barrows were sedated with a telazol-ketamine-xylazine cocktail (6.0, 8.0, and 4.0 mg/kg of BW, respectively, i.m.) and then maintained under anesthesia with halothane. Once anesthetized, approximately 15 cm of plastic tubing (i.d., 0.76 mm; o.d., 2.29 mm; Norton, Akron, OH) was inserted into the jugular vein toward the heart and then s.c. routed around the neck to an exit point just below the base of the neck using a custom-designed trocar (length, 31 cm; i.d., 4.0 mm). Approximately 0.3 m of tubing was allowed to extend from the exit point. The entire catheter was flushed with a sterile 0.9% saline solution, filled with a heparin solution (0.02%), and then sealed. The external portion of the tubing was then wrapped in a piece of cloth and attached to the back of the pig just below the base of the neck using rubber cement. After the catheterization procedure, the pig was returned to its home pen and observed for signs of recovery. Catheters were flushed once daily until d 8 by drawing blood through the tube, flushing with saline, filling with a heparin solution, and sealing the tubing.

Blood was collected from all barrows on d 8 of the study at approximately 0730 h. Once blood was collected (approximately 0845 h), barrows in the control group were fed as normal, and the feed-deprived barrows continued their feed deprivation. Blood was collected from all barrows at 6, 12, 24, and 36 h after 0800 h of d 8, when the feed-deprived group would normally have received their daily access to feed. The bleeding times corresponded with 1400 and 2000 h on d 8 and 0800 and 2000 h on d 9. Each barrow in which the catheter became nonfunctional had the catheter removed and was sampled via jugular venipuncture into an EDTA and a serum vacuum tube (Vacutainer, Becton, Dickinson and Co., Franklin Lakes, NJ).

During each blood collection, approximately 15 mL of blood was collected, 3 mL of which was collected for serum and allowed 1 h to clot at 4°C. The remaining 12 mL was collected into a syringe preloaded with 0.3 mL of a 7.5% EDTA solution. Two milliliters of whole blood with EDTA was combined with 1 mL of aprotinin solution (0.2 mg/mL, A1153, Sigma-Aldrich, St. Louis, MO) in a glass tube for analysis of glucagon. All collected blood was put on ice until centrifuged (1,600 × *g* for 15 min) within 1 h of collection, and then serum or plasma was harvested and frozen at –80°C until analysis.

Hormone and Metabolite Assays. Concentrations of hormones and metabolites were analyzed for each time point. Plasma insulin concentrations were assayed in duplicate using a commercially available RIA kit (TKIN1, Diagnostic Products Corp., Los Angeles, CA). Intrasample CV were less than 10%. Cross-reactivity of the insulin antibody, as determined by the manufacturer, was as follows: proinsulin, 20%; C-peptide and glucagon, 0%. Assays were performed using the overnight incubation protocol. Precision and accuracy of the assay were evaluated using quality controls (CON6, Diagnostic Products Corp.) and resulted in an intraassay CV of 3%. All samples were run in a single assay.

Plasma glucose concentrations were assayed in duplicate using a commercially available, enzymatic-colorimetric kit (1070, Stanbio Laboratory, Boerne, TX). Samples with an intrasample CV greater than 10% were rerun. Precision and accuracy of the assays were evaluated using a quality control (Ser-T-Fy I, Stanbio

Laboratory) containing 87 mg of glucose/dL, which resulted in intra- and interassays CV less than 15%.

Plasma creatinine concentrations were assayed in duplicate using a commercially available, enzymatic-colorimetric kit (500701, Cayman Chemical Co., Ann Arbor, MI). Samples were rerun when the intrasample CV were greater than 10%. Interference of the enzymatic substrate with other plasma components has not been assessed by the manufacturer. Assays were performed according to the manufacturer's specifications, with the exception of the last step (addition of 5 μL of picric acid), which we found to cause excessive amounts of precipitate. Precision and accuracy of the assays were evaluated using a quality control (691, Bio-Rad, Irwin, CA) and resulted in intra- and interassay CV less than 12% and 10%, respectively.

Plasma lactate concentrations were assayed in duplicate using a commercially available, enzymatic-colorimetric kit (Lactate, University of Buffalo, Buffalo, NY). Samples were rerun when the intrasample CV were greater than 10%. Assays were performed according to the manufacturer's specifications in addition to a 1:20 dilution of sample plasma that was necessary to bring the sample concentrations within a linear range of the standards. Precision and accuracy of the assays were evaluated using a quality control (691, Bio-Rad) and resulted in intra- and interassay CV of less than 14% and 25%, respectively.

Plasma cortisol concentrations were assayed in duplicate using a commercially available RIA kit (CA1549, DiaSorin Inc., Stillwater, MN). Samples with an intrasample CV greater than 10% were excluded from analysis. The cortisol antibody was found by the manufacturer to cross-react with the following: prednisolone, 77%; 6-methylprednisolone, 43%; 11-deoxycortisol, 6.3%; and corticosterone, <0.4%. Precision and accuracy of the assay were evaluated using quality controls (CON6, Diagnostic Products Corp.) and resulted in intra- and interassay CV less than 9% and 28%, respectively.

Total NEFA concentrations were assayed from serum in duplicate using a commercially available, enzymatic-colorimetric kit (NEFA-C, Wako Diagnostics, Richmond, VA). Samples were rerun when the intrasample CV was greater than 10%. Precision and accuracy of the assay were evaluated using a quality control (410-00101, Wako Diagnostics) and resulted in intra- and interassay CV of less than 12% and 9%, respectively.

Serum concentrations of β-hydroxybutyrate were assayed in duplicate using a commercially available, enzymatic-colorimetric kit (2440, Stanbio Laboratory). Samples were rerun when the intrasample CV was greater than 10%. Precision and accuracy of the assay were evaluated using a set of quality controls (2460, Stanbio Laboratory), which resulted in intra- and interassay CV of less than 14%.

Exp. 2: Behavioral Profile

Animals. For the behavioral profile, a feed deprivation protocol similar to that used for barrows in the physiological profile was used for an additional group of 16 group-housed barrows (n = 16; 88.8 ± 0.13 kg of BW; Hampshire-Duroc × Yorkshire-Landrace) in a single replicate. The major difference between the physiology and behavioral experiments was the absence of catheterization and blood collections as performed in the former; otherwise, all aspects of the studies including environment, source of barrows, and care were the same. Although behavioral data could have been collected during the physiological profile, the blood collection process and snaring of the barrows to collect blood when the catheters became nonfunctional was seen as a confounding factor. Thus, a completely separate but similar experiment was conducted.

As with the physiological profile, barrows were randomly assigned to the feed-deprived (n = 8) or control (n = 8) treatments applied on d 8 of the study. Four additional barrows served as extras to replace any barrows that had to be removed from the study. Each treatment was assigned to 2 of these 4 spare barrows, and they were treated in the same fashion, though not observed.

Behavioral Observations. Live behavioral observations of all barrows in each treatment were conducted by 4 trained observers for 4.5-min sessions at times that coincided with blood collections during the physiological profile of Exp. 1 (d 8: 0700, 1300, and 1900 h; d 9: 0700 and 1900 h). The 4.5-min sessions allowed the observers to more easily track their progress during the observations by maintaining a 5-min interval for each observational session. At the beginning of each time, the 4 investigators entered the animal room and stationed themselves at specific pens (1, 8, 9, 16) as indicated in Figure 1. Each observer had a pen, timer, clipboard, and preprinted data sheet for each animal and that was divided into 5-s intervals for the 4.5-min observation period.

Observations began with an initial 5-min period, during which no observations were taken, allowing the barrows to acclimate to the observer's presence (time = −5.0 min). At time = 0.0 min, observers began recording a single animal's specific behavior and posture every 5 s using the described ethogram (Table 1) for the 4.5-min observation. After the observation, each observer moved to the next pen in a clockwise fashion, as labeled in Figure 1, and began the next set of observations at time = 5.0 (0.0) min on a second data sheet. Observers continued in this fashion until each observer had observed all study barrows (total time = 80.0 min). Under this protocol, 2 barrows in each treatment were being observed at all times (with the exception of the 0.5-min interval between observations), and each animal was observed for a total of 18 min (4.5 min × 4 sessions) within each 80-min observation period.

The pen that each observer began observations for a particular time point in and the order in which subsequent pens were observed was randomized. To enhance interobserver consistency, a training session for observers was also conducted several days before the observa-

Table 1. Ethogram of observed behaviors

Behavior	Description
Posture	
Standing	Body supported by 4 legs (either with or without motion)
Sitting	Body supported by front legs, hind legs, and flat on the ground
Kneeling	Front legs bent, hind legs stretched vertically
Lying	
Laterally	Lying on the side with 3 or more legs stretched horizontally
Ventrally	Lying on the sternum, belly, or both with legs either under the body or the front legs outstretched
Activity	
Inactive[1]	No visible motion (with the exception of head turning)
Oral manipulations	
Floor	The mouth or snout is in contact with the floor and the top of the snout is below the bottom bar of the pen
Pen	The mouth or snout is in contact with any pen fixture including the nipple waterer (if not involved in drinking)
Drinking	Any contact of the snout with the nipple waterer
Walking[2]	Motion of the legs that results in movement of the head's position
Scratching	Rubbing the body against a portion of the pen or use of hind legs to rub a portion of the body
Escape attempt	Both front legs on pen bars
Sham chewing	Chewing motion without any substrate within jaws
Bar biting	Pen bars are within the animal's mouth
Licking	
Floor	Tongue extended and in contact with the floor
Pen bars	Tongue extended and in contact with the pen bars
Position change	Any motion involved in the transition between 2 classes of posture

[1]Could occur in any posture.
[2]Includes pivoting on front or rear legs.

tions began. Lastly, during the 7-d adaptation period, barrows were acclimated to the presence of the observer by having a single observer enter the animal room in the morning before feeding (~0730 h) and after feeding (~1200 h), during which the observer would slowly circle the room for a 5-min period. The total number of occurrences for each behavior recorded during the 4 investigators' observations was summed and used as the response variable for analysis.

Statistical Analysis. Hormone and metabolite concentrations and behavioral data were examined to ensure patterns of constant variance and a normal distribution. When these conditions were not met, the data were transformed by taking the log or square root, as needed. The resulting data were analyzed using the MIXED procedure (SAS Inst. Inc., Cary NC), where metabolite and hormone concentration or the summed number of behavioral counts of an individual barrow was the response variable, and blood collection and observation time, treatment, and their interactions were the independent variables. Replicate and blood collection method (via catheter or venipuncture) was also included in the model for the physiological profile. Animal was treated as a random factor and nested in treatment (replicate × treatment for the physiological data). Multiple observations on the same pig over the deprivation period were accounted for using the repeated option and a spatial covariance structure for the different

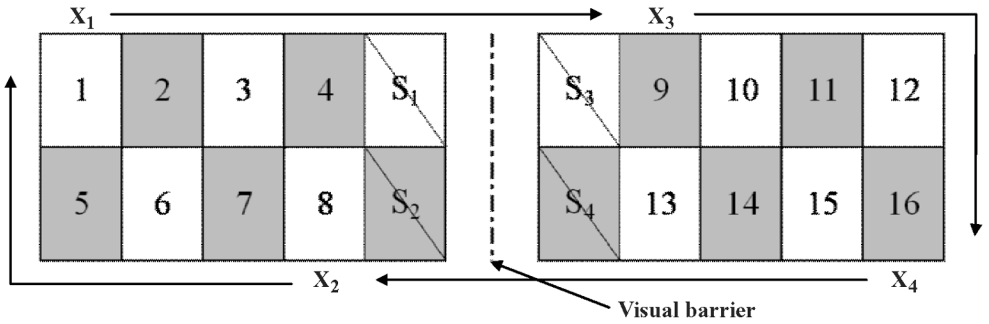

Figure 1. Barrows were maintained in individual pens and grouped by treatment. A visual barrier was present as indicated to prevent treatments from viewing one another. Barrows in pens 1 through 8 received the control treatment, whereas pens 9 through 16 received the feed-deprived treatment and were food-deprived for 36 h. Pens S1 through S4 served as substitute barrows and were not observed. Investigators (Xi) began at an initial pen, where observations were made for 4.5 min, and thereafter they moved to a second pen (as indicated by the clockwise arrows) and began a second set of observations. Observations continued until all 4 investigators had observed all barrows.

Table 2. Least squares means of physiological measures associated with treatment[1]

Measure	Treatment[2]		SE	*P*-value
	Control	Feed-deprived		
Cortisol,[3] μg/dL	2.0	2.1	0.3	0.43
Creatinine, mg/dL	2.31	2.46	0.09	0.25
Glucose, mg/dL	87.6	80.2	1.2	<0.001
Lactate,[3] μ*M*	4.5	4.2	0.6	0.41

[1]Blood samples were taken from control or feed-deprived animals at 21, 27, 33, 45, and 57 h of feed deprivation via jugular catheter or venipuncture. Catheters that became nonfunctional requiring venipuncture included the following: 21 h feed-deprived, 4; control, 4); 27 h (feed-deprived, 5; control, 5); 33 h (feed-deprived, 8; control, 7); 45 h (feed-deprived, 9; control, 11); and 36 h (feed-deprived, 9; control, 11). Treatment × sampling time interactions were not detected ($P > 0.10$).

[2]Animals were acclimated to free-access feed for a 3-h feeding period. After an 8-d period of adaptation, control animals continued the feeding regimen, whereas feed-deprived animals were feed-deprived for 57 h.

[3]Statistical analyses were performed on log-transformed data. Least squares means were calculated from transformed data and then back-transformed for presentation of data. Variance of data that required transformation is represented by the width of the 95% confidence interval.

lengths between sample times. Least squares means were calculated using the LSMEANS option of PROC MIXED, and differences between means were determined using the PDIFF option. To adjust for multiple comparisons in the treatment × time interaction, the 2 treatments were compared at each time point using a Tukey's comparison, where $\alpha = 0.01$. For data that required a transformation, the resulting least squares means were back-transformed for data presentation. Variance for the treatment means of transformed data was computed as the width of a 95% confidence interval around the transformed mean (Bland and Altman, 1996) and then back-transformed.

RESULTS

Exp. 1: Metabolic and Hormonal Measures

Metabolic and hormonal responses measured in the deprived treatment followed a pattern suggesting a catabolic state compared with control barrows (Table 2). Glucose concentrations of feed-deprived barrows were lower ($P < 0.001$) than in controls throughout the 57-h feed deprivation period.

Treatment × time interactions were observed ($P < 0.05$) for measures of insulin, β-hydroxybutyrate, and NEFA (Table 3). Insulin concentrations of control barrows were greater than feed-deprived barrows at all time points following the initial 21-h blood collection ($P < 0.01$). Concentrations of the ketone body β-hydroxybutyrate were similar between treatments through the 33-h blood collection ($P = 0.09$), whereafter concentrations of the feed-deprived treatment were greater ($P < 0.01$). Concentrations of NEFA in feed-deprived bar-

Table 3. Least squares means of physiological measures associated with the interaction of time and treatment

Measure,[2] units	Feed deprivation,[1] h											
	21			27			33			45			57		
	Control	Feed-deprived	Pooled SE	Control	Feed-deprived	Pooled SE	Control	Feed-deprived	Pooled SE	Control	Feed-deprived	Pooled SE	Control	Feed-deprived	Pooled SE
Insulin, pmol/L	4.0	5.2	1.4	14.0[a]	2.3[b]	2.5	6.8[a]	2.7[b]	1.5	6.8[a]	2.8[b]	1.4	5.4[a]	2.0[b]	1.1
β-hydroxybutyrate, m*M*	0.17	0.17	0.01	0.19	0.18	0.01	0.18	0.18	0.01	0.18[a]	0.20[b]	0.01	0.17[a]	0.23[b]	0.01
NEFA, mEq/L	0.30	0.31	0.03	0.29[a]	0.50[b]	0.04	0.30[a]	0.63[b]	0.04	0.31[a]	0.56[b]	0.04	0.29[a]	0.66[b]	0.05

[a,b]For each time point, means within a time point differ ($P < 0.01$).

[1]Treatment × time interactions were detected ($P < 0.05$). For the experiment, animals were acclimated to free access to feed for a 3-h feeding period. After an 8-d period of adaptation, control animals continued the feeding regimen, whereas feed-deprived animals were feed-deprived for 57 h. Catheters that became nonfunctional requiring venipuncture included the following: 21 h (feed-deprived, 4; control, 4); 27 h (feed-deprived, 5; control, 5); 33 h (feed-deprived, 8; control, 7); 36 h (feed-deprived, 9; control, 11); and 45 h (feed-deprived, 9; control, 11).

[2]Statistical analyses were performed on log-transformed data. Least squares means and the width of the 95% confidence interval around the mean were calculated and then back-transformed for presentation in this table.

Table 4. Least squares means of behavioral measures associated with treatment

	Treatment[1]			
Activity	Control	Feed-deprived	SE	*P*-value
Bar biting,[2] counts per 18 min	3.2	4.5	3.6	0.454
Changing position,[2] counts per 18 min	2.1	3.5	1.3	0.052
Oral-nasal pen manipulations,[2] counts per 18 min	15.5	26.7	12.4	0.053
Scratch,[2] counts per 18 min	0.3	0.5	0.3	0.385
Sham chewing, counts per 18 min	7.3	9.7	2.0	0.405
Walking,[2] counts per 18 min	3.4	6.1	2.9	0.059

[1]Animals were either fed normally or feed-deprived and observed for behavioral activity at 21, 27, 33, 45, and 57 h.
[2]Statistical analyses were performed on log-transformed data. Least squares means and the width of the 95% confidence interval around the mean were calculated and then back-transformed for presentation in this table.

rows were greater than the control treatment for all collections after 21 h ($P = 0.001$). Glucose concentrations were not affected by the interaction of dietary treatment and time ($P > 0.10$), although results may have been confounded by a tendency for a replicate × treatment effect ($P = 0.07$). No treatment or treatment × time differences were found for the responses of cortisol, creatinine, or lactate ($P > 0.12$).

Exp. 2: Behavioral Measures

Compared with control barrows, feed-deprived barrows performed more ($P = 0.05$) position changes, oral-nasal pen manipulations, and walking (Table 4). Likewise, deprived barrows were observed to be kneeling more often ($P = 0.03$) and lying lateral less often ($P = 0.05$). Barrows of the feed-deprived treatment were found to be inactive less ($P = 0.03$) but are discussed in terms of the treatment × time interaction found. All other behavior measurements were not affected by treatment ($P > 0.18$).

Analysis of behavioral observations for activity found treatment × time interactions resulted in more drinking at 27 h in control barrows ($P < 0.01$; Table 5). Barrows in each treatment were similarly inactive at all time points ($P > 0.19$), with the exception of at 45 h of deprivation, in which control barrows were observed to be inactive more often than feed-deprived barrows ($P < 0.01$; Table 5). A tendency existed for control barrows to lick their pens more often ($P = 0.02$; Table 5) at 27 h of deprivation. Tendencies also existed for control barrows to perform more oral-nasal floor manipulations at 27 h ($P = 0.04$; Table 5), although this pattern was reversed at 45 and 57 h of deprivation, in which feed-deprived barrows tended to perform the behavior more often ($P < 0.03$; Table 5). Barrows in the feed-deprived treatment were found to kneel more often at 21 h of deprivation ($P < 0.01$; Table 6). Barrows in the control treatment were found lying lateral more often at 57 h ($P < 0.01$), whereas a tendency for increased lying laterally was found at 45 h ($P = 0.04$; Table 6). Lying sternal was found to occur more often in feed-deprived barrows at 57 h ($P < 0.01$; Table 6). At 45 h of deprivation, feed-deprived barrows were found to stand more often ($P < 0.01$; Table 6). Tendencies existed for control barrows to sit and stand more often at 21 and 27 h of deprivation, respectively ($P = 0.04$; Table 6).

DISCUSSION

The current project sought to utilize the knowledge of physiological and behavioral responses that exists following metabolic challenges to generate specific profiles during periods of feed deprivation that are likely to correlate with the hunger an animal is experiencing. Hunger is the term given to the internal energized state that is the predominant causal factor in food-seeking behavior (Toates, 1986). Hunger occurs on a spectrum ranging from feelings of starvation to satiety, with infinite gradations between these 2 extremes. The regulation of hunger is equally complex, and many systems have been proposed in which a single factor is responsible for inducing sensations of hunger (i.e., glucostatic theory; Le Magnen, 1985), although all have been incapable of explaining the many intricacies of hunger and the behavioral expression of feeding. Rather, hunger is more likely a product of overlapping central (Broberger, 2005) and peripheral (Stanley et al., 2005) systems that serve to regulate the hunger that an animal experiences. Previous efforts have attempted to quantify hunger of swine in a variety of the mentioned contexts, including the following: motivational (Lawrence et al., 1988, 1989; Lawrence and Illius, 1989), behavioral (Appleby and Lawrence, 1987; Terlouw et al., 1991; De Leeuw and Ekkel, 2004), and physiological (Douglas et al., 1998; Farmer et al., 1998) measures.

Our effort involved an alternative approach to characterizing hunger by imposing specific lengths of feed deprivation on animals and then identifying physiological and behavioral responses that might be associated with different severities of deprivation and possibly underlying hunger. Although previous efforts have taken a multidisciplinary approach to identifying states of hunger in given situations, our study is unique in that

we attempted to create specific gradations of hunger with increasing periods of feed deprivation. Given that increasing lengths of feed deprivation will correlate with transitive changes in hunger, resulting profiles can be used to assess how specific physiological and behavioral responses associate with hunger.

The physiological responses affected by feed deprivation suggest that food-deprived animals were in a catabolic state. As expected based on previous work (Barb et al., 1997), the reduced but stable plasma glucose concentrations of feed-deprived compared with control animals indicates the former had effectively adjusted to fasting, likely by increased use and production of alternative fuels for energetic needs. The suggestion that feed-deprived animals were mobilizing alternative energies is supported by the increased concentrations of serum NEFA and β-hydroxybutyrate, results that were similar to that of others (Simoes Nunes et al., 1987; Ruwe et al., 1991; Barb et al., 1997). Although decreased lactate and increased creatinine concentrations were expected as further indications of metabolic adaptation, no changes in these measures were observed.

No differences between control and feed-deprived animals were observed in cortisol concentrations in the current study. Increased release of glucocorticoids occurs during both metabolic and psychological challenges (Ewing et al., 1999) and thus can indicate a wide range of potential stressors. The absence of a treatment effect in terms of cortisol concentrations may suggest that the applied feed deprivation did not pose a significant nutritional or metabolic challenge to the animals. However, changes in other physiological (i.e., β-hydroxybutyrate, NEFA) and behavioral measures (i.e., inactivity) indicate that the feed deprivation was at least minimally offensive to physiological homeostasis and comfort levels. Additionally, cortisol concentrations have been shown to increase in similar scenarios, in which weanling barrows were subjected to 72 h of feed deprivation (Salfen et al., 2003), thus alternative explanations for the lack of a treatment effect should be considered. For instance, the dietary regimen used in the current study provided 3 h of ad libitum access to feed and may have been inadequate to sustain satiety for the entire day. Although our reasoning for this regimen was to standardize levels of hunger across animals, control animals may have been experiencing a level of hunger that resulted in elevated cortisol concentrations. Despite this possibility, cortisol concentrations in the current study did not seem elevated compared with findings by others (Fernandez et al., 1995; Marchant-Forde et al., 2003). Another factor to consider is that in multiple instances, blood collection had to be taken via venipuncture when catheters became nonfunctional, although statistical analysis showed that collection method was not a factor in collection, so it's unlikely that this affected our findings. The possibility also exists that changes in cortisol concentration between treatments may have been different at times

Table 5. Least squares means of activity measures associated with the interaciton of time and treatment

	Feed deprivation,[1] h											
	21			27			33			45			57		
Measure	Control	Feed-deprived	Pooled SE	Control	Feed-deprived	Pooled SE	Control	Feed-deprived	Pooled SE	Control	Feed-deprived	Pooled SE	Control	Feed-deprived	Pooled SE
Drinking, counts per 18 min	3.1	2.7	1.3	9.6[a]	1.6[b]	1.3	1.1	2.8	1.3	1.1	2.6	1.3	1.4	2.6	1.3
Inactivity, counts per 18 min	79.4	69.6	12.5	115.2	131.1	12.9	181.54	157.5	12.9	118.6[a]	53.3[b]	12.5	185.5	153.0	12.5
Licking floor,[2] counts per 18 min	1.5	4.3	3.6	1.3	0.4	1.8	1.3	1.5	2.3	6.7	2.8	5.3	0.9	1.6	2.1
Licking pen,[2] counts per 18 min	1.6	1.0	1.5	0.3[c]	2.1[d]	1.4	0.6	0.4	1.0	1.7	1.1	1.5	0.9	2.5	1.7
Oral-nasal floor manipulations,[2] counts per 18 min	38.7	45.2	28.9	37.4[c]	16.9[d]	20.0	4.6	5.8	4.3	27.9[c]	63.6[d]	31.4	2.2[c]	6.3[d]	3.5

[a,b]For each measure, means within a time point differ ($P < 0.01$).
[c,d]For each measure, means within a time point differ ($P < 0.05$).
[1]Treatment × time interactions were detected ($P < 0.05$). Animals were acclimated to free access to feed for a 3-h feeding period. After an 8-d period of adaptation, control animals continued the feeding regimen, whereas feed-deprived animals were feed-deprived for 57 h.
[2]Statistical analyses were performed on log-transformed data. Least squares means and the width of the 95% confidence interval around the mean were calculated and then back-transformed for presentation in this table.

Table 6. Least squares means of posture associated with the interaction of time and treatment

	Feed deprivation,[1] h											
	21			27			33			45			57		
Measure	Control	Feed-deprived	Pooled SE	Control	Feed-deprived	Pooled SE	Control	Feed-deprived	Pooled SE	Control	Feed-deprived	Pooled SE	Control	Feed-deprived	Pooled SE
Kneeling, counts per 18 min	1.6[a]	6.4[b]	0.8	1.9	1.8	0.9	1.3	1.4	0.9	2.4	4.5	0.8	0.3	1.0	0.8
Lying lateral,[2] counts per 18 min	2.5	0.1	4.0	0.0	1.8	3.4	13.1	17.4	29.7	4.1[c]	0.2[d]	5.4	104.2[a]	5.7[b]	97.9
Lying sternal, counts per 18 min	98.5	83.6	17.5	112.6	138.1	18.13	151.1	129.4	18.1	125.5	86.5	17.5	75.5[a]	137.0[b]	17.5
Sitting, counts per 18 min	9.3[c]	2.5[d]	2.3	3.8	3.1	2.4	1.0	4.9	2.4	1.5	1.8	2.3	1.3	6.3	2.3
Total lying, counts per 18 min	106.0	83.8	14.4	106.9[a]	156.8[b]	14.4	190.8	167.0	14.4	142.9[a]	86.8[b]	14.43	189.1	161.9	14.4
Standing, counts per 18 min	103.1	127.4	14.5	102.2[c]	58.4[d]	14.9	22.3	46.8	14.9	73.3[a]	127.0[b]	14.5	29.4	50.9	14.5

[a,b]For each measure, means within a time point differ ($P < 0.01$).

[c,d]For each measure, means with a time point differ ($P < 0.05$).

[1]Treatment × time interactions were detected ($P < 0.05$). Animals were acclimated to free access feed for a 3-h feeding period. After an 8-d period of adaptation, control animals continued the feeding regimen, whereas feed-deprived animals were feed-deprived for 57 h.

[2]Statistical analyses were performed on log-transformed data. Least squares means and the width of the 95% confidence interval around the mean were calculated and then back-transformed for presentation in this table.

when blood was not collected (i.e., shortly after the anticipated meal was not provided to feed-deprived animals).

From a purely metabolic standpoint, the stability of glucose in feed-deprived animals during the current study, albeit reduced from control animals, suggests feed-deprived animals were able to maintain an adequate level of glucose as expected. Humans are able to mobilize endogenous resources and metabolically adapt to periods of food deprivation extending over 60 d (Cahill et al., 1966; Owen et al., 1969), utilizing increased fatty acid production within the first several days, followed by increased ketone body production. Similar patterns of substrate use have been shown in pigs fasted for periods of 4 d using indirect calorimetry (Chwalibog et al., 2004, 2005) and confirmed by endocrinological measures (Barb et al., 1997). Results from the current study followed this pattern as shown by increased NEFA within 27 h of deprivation and increased ketone body concentration at 45 and 57 h. The measurement of NEFA in the current study was a measure of total NEFA and did not take into account individual fatty acid types; however, we can estimate that the composition of total NEFA in the fasted pig was 75% palmitic, steric, and oleatic acid (Freeman et al., 1970). Assuming a NEFA pool with this composition, NEFA would provide approximately 1,788 kcal·mol^{-1} (256.4 g × 9.3 kcal·g^{-1} × 75%). In this scenario, the increased NEFA concentrations in a feed-deprived animal would provide an energy yield that exceeded the calories provided by glucose and NEFA in control animals at all time points, suggesting the challenge of the deprivation period was within the metabolic adaptive capacity of the animal. Our estimates do not consider several factors, including variation in the metabolism of individual circulating NEFA type (Freeman et al., 1970; Lindsay, 1975) and severity of fasting (Freeman et al., 1970) or estimations of energetic costs associated with AA deamination or gluconeogenesis. Thus, any conclusions regarding the change in specific energetic substrates will need to be verified by in vivo methods. Nonetheless, our results suggest that feed-deprived animals were able to maintain adequate levels of energy during the fasting period. However, although animals were able to produce adequate levels of energy and could likely continue to do so for several weeks despite feed deprivation, this circumstance would also be likely to reduce or reverse weight gain, inhibit normal immune function, and reduce other measures associated with overall health. Any definitive assessments of welfare should include all relevant measures such as BW gain and immune function (Moberg, 1987; Dawkins, 1990) if longer periods of deprivation are used

In terms of behavioral responses, kneeling was greater in deprived animals at 21 h of deprivation, a time when neither treatment had been applied, thus the finding was unexpected. Examination of the kneeling data at 21 h revealed that the feed-deprived treatment contained an abnormally high count for 1 particu-

lar pig as observed by a single observer; however, the data point was not removed from analysis, because no other evidence suggested it was an outlier. Kneeling following 21 h of deprivation was not affected by treatment × time interactions. Behavioral responses also exhibited a strong diurnal pattern, with the greatest amount of total lying and inactivity tending to be performed at the nighttime observations (i.e., 33 and 57 h of deprivation). The effect of diurnal rhythm was expected and the reasoning for including the control treatment in our experimental design. Overall, behavioral results suggested that feed-deprived animals were more active. Our results of increased activity in feed-deprived animals agree with findings of others (Appleby and Lawrence, 1987) and suggest a pattern dependent on deprivation length. The comparatively reduced restfulness at 27 h of deprivation in control animals as manifested by decreased total lying and increased drinking may be related to that day's earlier feed provision, enhancing the motivation to remain active. A tendency existed for control animals to perform more oral-nasal floor manipulations at 27 h, although feed-deprived animals tended to perform licking the pen more. A possible explanation for the difference between observations of oral-floor manipulations and licking the pen is that oral-nasal floor manipulations were typically performed when the animal was standing, a notion supported by the decreased total lying in control animals at 27 h. Motivational states are dependent on both external and internal stimuli (Toates, 1986), thus it is interesting to consider whether the provision of feed to control animals decreased the desire to lay and perform restful behavior. In such a scenario, feed-deprived animals, without the stimulus of feed, may have experienced a rapid dissipation of feed motivation, possibly aided by the increased licking of the pen observed within the same time frame serving as an output for redirected feed motivation. In support of this conclusion, Terlouw et al. (1993) found ingestion of feed stimulated the performance of abnormal behaviors such as stereotypies and suggested that providing feed began a series of feedback loops leading to enhanced feed motivation. The effect was independent of the amount of feed provided. Although increased postfeeding abnormal behaviors are often considered an indication of hunger (Lawrence et al., 1993), our results and those of Terlouw et al. (1993) suggest that feed ingestion by itself may intensify feed motivation. Alternatively, the increased pen licking in feed-deprived animals at 27 h could be indicative of a high level of frustration. Additionally, because control animals had ad libitum access to feed during the 3-h morning feeding session, we would expect that any increased motivation to feed would be satisfied by continued intake, unless the period of free access to feed was not sufficient or a separate need to perform foraging behaviors existed. With these uncertainties remaining, conclusions regarding level of comfort during these periods cannot be made definitively. However, future efforts to alleviate hunger may find success in examining interval feeding schedules, or feeding every other day, which have been suggested to provide greater feelings of satiety over being fed once per day (Douglas et al., 1998).

Although there was little evidence to suggest differences in general activity at 27 h of deprivation, feed-deprived animals appeared to become more active as the severity of deprivation extended. Lying laterally (vs. sternally), which was greater in control animals at 57 h, has been shown to be the preferred position in pigs experiencing a deep period of sleep (i.e., later at night; Ekkel et al., 2003) and more associated with satiated animals (Zonderland et al., 2004). In further support of increased activity in feed-deprived animals at later stages of deprivation, control animals tended to be lying lateral more at 45 h of deprivation, and feed-deprived animals tended to perform more oral-nasal manipulations at 45 and 57 h of deprivation. The greater occurrence of oral-nasal manipulations was likely a result of increased feed motivation inducing foraging behaviors.

Interestingly, with the exception of increased drinking in control animals at 27 h of feed deprivation likely associated with the earlier meal, no other differences were observed between treatments. Increased drinking is often correlated with increased hunger in swine (Rushen, 1985; Robert et al., 1993) and has been suggested to result from an effort to distend the stomach or boredom (Robert et al., 1993). We expect that the greater activity seen during the 45- and 57-h periods of deprivation would be manifested at least partially in increased drinking. However, Robert et al. (1993) also observed a higher frequency of drinking associated with a lower frequency of rooting in control sows provided a restricted ration, thus the lack of increased drinking may be related to the increased oral-nasal floor manipulations observed.

The pairing of physiological and behavioral observations within a similar experimental framework provides a critical means to determine how responses in each measure relate to each other. In the period of deprivation ranging from 21 to 33 h, few differences were identified between treatments, with the exception that control animals appeared to lie less and drink more than feed-deprived animals, whereas at the 45 and 57 h observation times, the pattern of activity was reversed and feed-deprived animals were more active. In considering the physiological changes during the same time frame, animals are capable of maintaining adequate levels of energy for 12 h of deprivation through hepatic glycogenolysis (Genuth, 1998), after which oxidation of fat stores becomes the predominant energy source once adequate production capacity is reached (Chwalibog et al., 2004). Assuming that the briefer periods of feed deprivation (<33 h) represent a less severe metabolic challenge than the 45- and 57-h deprivation periods of the current study, it is possible that the reduced activity in feed-deprived animals seen at 27 h represents a period in which feed motivation did not

require a dramatic increase due to the efficiency of hepatic glycogenolysis. Animals in the current study had been acclimated to a 3-h period of ad libitum feed access in the morning. Thus, hepatic glycogen production was likely not dramatically increased until after the period when feed-deprived animals would have expected their daily ration at 21 h of deprivation (0800 h on d 8). In support of this possibility, other physiological indicators of a catabolic state were not different at that time. The increased occurrence of licking the pen at 27 h in feed-deprived over control animals may have represented an adaptive response or displaced feed motivation related to frustration rather than nutrition. The increased activity at 45 and 57 h in feed-deprived animals occurred during the observation times when the metabolic adaptations to feed deprivation had likely switched from its initial response of hepatic glycogenolysis to the more energetically productive process of fatty acid oxidation. The specific behavioral changes during these more severe metabolic adaptations may be a product of an increased sense of urgency or feed motivation that manifests as heightened activity. Alternatively, the occurrence of behavioral and metabolic changes between control and feed-deprived within the same time frame could be related to natural diurnal rhythms or simply correlated responses without a causal relationship. The increased activity in feed-deprived animals at later stages could also be explained by boredom that resulted when the primary event of excitement during the day (provision of feed) was no longer occurring (Lawrence and Terlouw, 1993). In support of our initial explanation that the behavioral changes of the 45 and 57 h are a product of underlying metabolic processes, the link between neural processes, feed motivation, and endogenous substrates is well recognized. Bindra (1959) reviewed the role of blood components, including metabolites, in influencing motivational states and concluded that responses are often attributed to a variety of stimuli. Toates (1980) considered the lipostatic and glucostatic models to explain the formation of behavioral responses during periods of energetic deprivation, although admitted the limitations of such models. Evidence also exists to support a role for NEFA and ketone bodies in appetite regulation (Scharrer, 1999). Although little evidence exists to support a role for NEFA and an increase in feed motivation, the period in which hepatic glycogen reserves become depleted and NEFA production becomes a principal source of energy as discussed earlier may represent a time frame in which the emotional experience of hunger becomes particularly intense, inducing the behavioral changes seen. In this scenario, although the animal's level of available energy is adequate, the perception of impending malnutrition may instill a feeling of intense hunger resulting from evolutionary mechanisms that seek to maintain metabolic stability (Dawkins, 1990). Furthermore, if the hunger experienced by animals at these particular deprivation lengths is correlated to specific physiological and behavioral responses, as proposed in the above discussion, it may offer a unique means to assess and objectively characterize the hunger that the animal is experiencing. For instance, a hypothetical assessment of restricted feeding in sows that revealed a profile of decreased insulin concentration, normal concentrations of NEFA and β-hydroxybutyrate, and high levels of inactivity would suggest the animals were experiencing a level of hunger comparable with 21 to 33 h of feed deprivation or less. Furthermore, the relative benefit of adding bulk such as straw or oat hulls to improve the satiating capacity of the diet could be judged by comparing the 2 diets in terms of the profile of hunger that they most resemble. Thus, the welfare of the sow can be improved by objectively determining its hunger and, if necessary, potential means to reduce it. The ability to make such decisions will require considerable additional experimental effort beyond the present work.

In conclusion, results of our work offer a unique assessment of different gradations or levels of hunger imposed. By including a wide array of measures, a broad picture that encompasses a range of responses and provides an objective framework to make assessments of welfare can be created. However, before such a methodology can be adopted to evaluate the effectiveness of diets, several key issues need to be resolved, including the effectiveness of the control feeding regimen in satiating an animal compared with the fasted treatment, diurnal rhythms underlying behavioral patterns, and potential alternatives to purposely increasing hunger (i.e., providing graded levels of energy). Lastly, the methodology would require testing over various settings to determine the consistency of methods across such settings (acute vs. chronic feed deprivation, growing pigs vs. mature sows, etc.).

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